Quantitative structure–activity studies of insect growth regulators: XVI. Substituent effects of dibenzoylhydrazines on the insecticidal activity to Colorado potato beetle *Leptinotarsa decemlineata*†

Yoshiaki Nakagawa,[1]* Guy Smagghe,[2] Soichi Kugimiya,[1] Kazunari Hattori,[1] Tamio Ueno,[1] Luc Tirry[2] and Toshio Fujita[1]

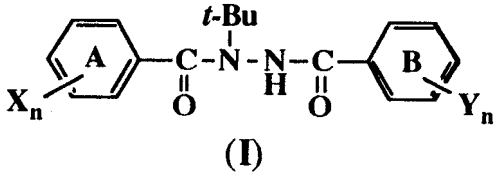
[1]*Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan*
[2]*Laboratory of Agrozoology, Department of Crop Protection, Faculty of Agricultural and Applied Biological Sciences, Gent University, B-9000 Gent, Belgium*

Abstract: Insecticidal activity against the Colorado potato beetle, *Leptinotarsa decemlineata*, was measured for a series of substituted *N-tert*-butyl-dibenzoylhydrazines, in which one of the benzoyl moieties closer to the *tert*-butyl group was fixed as being 2-chloro-substitued and the other variously substituted singly or doubly. The effects of substituents on the activity were quantitatively analysed using the classical quantitative structure–activity relationship (QSAR) procedure. The activity against the Colorado potato beetle increases with the molecular hydrophobicity. In addition, various types of steric effect are at work, depending upon the positions. Hydrogen-bonding acceptor substituents at the *para* position enhance the activity. There seem to be threshold (or optimum) values, albeit position-dependent, in the molecular hydrophobicity, above which the activity starts to decrease. This biphasic contribution of the molecular hydrophobicity to activity against coleopterous larvae is the most conspicuous difference in substituent effects from those found for similar compounds against lepidopterous pest insects, and may be the basis of the variations in the activity spectrum for certain compounds in this series. The introduction of bulkier substituents into the *meta*- and *para*-positions of the benzene ring, apart from the *tert*-butyl group, is unfavorable to activity. LD_{50} values against Colorado potato beetle larvae of methoxyfenozide (RH-2485) and tebufenozide (RH-5992) were in the order of 10^{-7} mol per insect, whereas those of RH-5849, and halofenozide (RH-0345) were very low, 10^{-9}–10^{-10} mol per insect being selective to the coleopterous larvae.

Keywords: tebufenozide (RH-5992); methoxyfenozide (RH-2485); halofenozide (RH-0345); RH-5849; QSAR; *Leptinotarsa decemlineata*; larvicidal activity

1 INTRODUCTION

Among nonsteroidal ecdysone agonists, *N-tert*-butyl-*N*,*N*′-dibenzoylhydrazine analogs, tebufenozide (RH-5992; Fig 1, **I**: X_n=3,5-$(CH_3)_2$, Y_n=4-C_2H_5) has recently been marketed as a novel type of insecticide to control lepidopterous pests.[1,2] At present, a related analog, methoxyfenozide (RH-2485, **I**: X_n=3,5-$(CH_3)_2$, Y_n=2-CH_3-3-OCH_3) is under development, being highly effective for the selective control of Lepidoptera, with a low toxicity profile towards mammals, birds and fishes, as well as towards non-target arthropods such as insect pollinators, predators, and parasitoids.[3] Because of



(I)

Figure 1. General structure of *N-tert* - butyl - *N*,*N*′-dibenzoylhydrazines.

* Correspondence to: Y Nakagawa, Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
† Part XV: Smagghe G *et al*, Comparative ecdysteroid action of ring-substituted dibenzoylhydrazines in *Spodoptera exigua*. *Arch Insect Biochem Physiol* **41:** 42–53 (1999)
Contract/grant sponsor: Ministry of Education, Science and Culture, Japan; contract/grant number: 10161207
(*Received 25 January 1999; accepted 10 May 1999*)

their mode of action, these compounds are applicable to insect species resistant to conventional neurotoxic insecticides such as pyrethroids and organophosphates.

Whereas tebufenozide is highly potent against lepidopterous pests, but only poorly against Coleoptera,[4] RH-5849 (**I**: X_n=H, Y_n=H) is toxic to Lepidoptera as well as to Coleoptera such as the Colorado potato beetle, *Leptinotarsa decemlineata* Say.[5] Halofenozide (RH-0345; **I**, X_n=H, Y_n=4-Cl), being toxic also against Lepidoptera, has a broad spectrum somewhat similar to that of RH-5849.[6,7] To explain the difference in the toxicity spectra between tebufenozide and RH-5849 against Lepidoptera and Coleoptera, the pharmacokinetics and metabolic detoxication mechanisms of these compounds were examined and compared using radio-labeled isotopes, but no significant difference was observed in such mechanisms between these two compounds.[4,5]

Previously, we have quantitatively analyzed the substituent effects for dibenzoylhydrazines on their in-vivo and in-vitro activities against the rice stem borer, *Chilo suppressalis* Walker[8–12] using quantitative structure–activity relationship (QSAR) procedures[13,14] to gain an insight into the molecular mechanism of the activity. The QSAR results indicate that the higher the molecular hydrophobicity, the higher the activity, but the bulkier the substituents introduced at any position of the A- and B-rings in the structure (**I**), the more unfavorable this is to the activity.[8,9] The optimum or the least unfavorable substitution patterns are to introduce hydrophobic but not bulky substituents into *meta* positions of the A-ring and the *para* position of the B-ring, as well as hydrophobic electron-withdrawing substituents into one of the *ortho* positions of the A-ring. We have also compared QSAR results for the insecticidal activity of 2-Cl analogs (**I**: X_n=2-Cl) having various substituents at the B-ring moiety between two lepidopterous insect species, rice stem borers and beet armyworms (*Spodoptera exigua* Hübner).[15] Effects of substituents in the B-ring moiety are very similar between the two insect species. Moreover, the very high potency of tebufenozide and RH-2485 to lepidopterous insects is well predicted by the QSAR analyses.

In this study, we measured the insecticidal activity of 2-Cl (in the A-ring) derivatives (**I**: X_n=2-Cl) having various substituents in the B-ring moiety (Y_n) against Colorado potato beetle larvae. The substituent effect in the B-ring was quantitatively analyzed using substituent parameters. The molecular hydrophobicity and its optimum, as well as the steric effect of subsituents, were very important in governing the activity against Colorado potato beetle larvae, depending upon substituent positions and substitution patterns. The insecticidal activity of RH-5849, tebufenozide, halofenozide and methoxyfenozide was also measured against Colorado potato beetle larvae and the physicochemical meaning for the low toxicity of tebufenozide and methoxyfenozide against Coleoptera is discussed.

2 MATERIALS AND METHODS

2.1 Compounds

Most of the compounds (Table 1, **1–33, 36–46**) were of samples the same as those used in our previous study.[9] Two compounds **34** (4-$COCH_3$) and **35** (4-SO_2CH_3) were newly synthesized conventionally.[8,9,16] The final compounds and intermediates were prepared following the typical procedures shown in Fig 2. The intermediates and final compounds were purified by either recrystallization or column chromatography on a Wakogel C-200 (Wako Pure Chemical Industries, Ltd, Osaka, Japan). Their structures were confirmed by [^{1}H]NMR and elemental analysis. [^{1}H]NMR spectra were recorded on a Bruker AC-300 NMR spectrometer at 300 MHz in deuterochloroform ($CDCl_3$) with tetramethylsilane as the internal standard. Melting points of the compounds were measured with a Yanaco melting point apparatus (Yanaco, Kyoto, Japan) and are uncorrected. Technical grade samples of tebufenozide (**47**) and methoxyfenozide (**48**), RH-5849 (**49**), halofenozide (**50**), were the kind gift of Dr G R Carlson (Rohm and Haas Research Laboratories, Spring House, PA, USA).

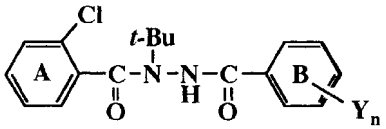
2.1.1 N-tert-Butyl-N′-(9-fluorenylmethoxycarbonyl) hydrazine (Step A)

tert-Butylhydrazine hydrochloride (10.0 g, 80.3 mmol) and sodium bicarbonate (6.50 g, 77.4 mmol) were suspended in dioxane + water (2 + 1 by volume; 100 ml), into which 150 ml of dioxane containing *N*-(9-fluorenylmethylcarbonyl)succinimide (Fmoc-Osu; 25.0 g, 74.1 mmol) was added dropwise while stirring in an ice bath. After stirring overnight at room temperature, the solvent was evaporated. The residue was triturated with ether, and the ether solution was washed with saturated brine. After drying the ether layer over anhydrous magnesium sulfate, the solvent was evaporated to give a colorless solid residue. The solid was manipulated with a mixture of hexane and ethyl acetate to afford *N-tert*-butyl-*N′*-(9-fluorenylmethoxycarbonyl)hydrazine (20.8 g) as a colorless powder (yield 83.6%).

2.1.2 N-tert-Butyl-N-(2-chlorobenzoyl)-N′-(9-fluorenylmethoxycarbonyl)hydrazine (Step B)

Triethylamine (10 ml) and 2-chlorobenzoyl chloride (4.00 g, 22.9 mmol) in anhydrous ether (80 ml) were simultaneously added dropwise to an anhydrous ether solution (100 ml) containing *N-tert*-butyl-*N′*-(9-fluorenylmethoxycarbonyl)hydrazine (6.32 g, 20.4 mmol) with stirring in an ice bath. After stirring overnight, methanol (50 ml) and ethyl acetate (70 ml) were added to the reaction mixture. The solution was washed with 1 M hydrochloric acid and brine, and the organic layer was then dried over anhydrous magnesium sulfate. After evaporating the solvent, the solidified residue was treated with a mixture of hexane and ethyl acetate to afford *N-tert*-butyl-*N*-(2-chlorobenzoyl)-*N′*-(9-

Table 1. Larvicidal activity of substituted dibenzoylhydrazines against last-instar larvae of *Leptinotarsa decemlineata* and physicochemical parameters



Compound		pLD_{50} (*mmol per insect*)				Substituent parameters				
			Calc[b]							
No	Y_n	*Obs*[a]	*Eqn* (5)[c]	*Eqn* (7)[d]	*log P*[e]	$\sum\Delta B_5^{ortho}$	$\sum E_s^{ortho\ f}$	$\sum E_s^{metal\ f}$	$E_s^{para\ f}$	*HB*
1	H	5.46±0.15 (5)	5.27	(4.84)	2.59[g]	0.00	0.00	0.00	0.00	0
2	2-F	5.93±0.18 (3)	5.44	(4.94)	2.63[g]	0.35	−0.32[h]	0.00	0.00	0
3	2-Cl	5.71±0.18 (4)	5.72	(5.08)	2.75[g]	0.80	−0.98[h]	0.00	0.00	0
4	2-Br	5.62±0.12 (2)	5.93	(4.89)	2.91	0.95	−1.12[h]	0.00	0.00	0
5	2-I	4.96±0.02 (2)	(6.18)	4.72	3.11	1.15	−1.44[h]	0.00	0.00	0
6	2-CF_3	6.33±0.10 (2)	6.28	(5.41)	3.02	1.61	−2.46[h]	0.00	0.00	0
7	2-NO_2	5.27±0.21 (3)	5.30	(6.72)	1.99	1.44	−1.65[h]	0.00	0.00	0
8	2-CH_3	6.24±0.06 (2)	5.96	(4.91)	2.91[g]	1.04	−1.16[h]	0.00	0.00	0
9	2-C_6H_5	4.98±0.03 (2)	(7.15)	4.85	3.77	2.11	−3.82	0.00	0.00	0
10	2-OCH_3	5.98±0.10 (2)	5.89	(5.42)	2.37	2.07	−0.40[h]	0.00	0.00	0
11	2-SCH_3	6.21±0.03 (2)	6.38	(5.02)	2.84	2.26	−1.14[h]	0.00	0.00	0
12	3-F	5.58±0.07 (2)	5.38	(4.59)	2.88[g]	0.00	0.00	−0.32[h]	0.00	0
13	3-Cl	6.09±0.15 (3)	5.64	(4.06)	3.49[g]	0.00	0.00	−0.98[h]	0.00	0
14	3-Br	5.66±0.06 (2)	5.69	(3.95)	3.62	0.00	0.00	−1.12[h]	0.00	0
15	3-I	4.03±0.08 (3)	(5.77)	3.77	3.87	0.00	0.00	−1.44[h]	0.00	0
16	3-CF_3	3.34±0.14 (3)	(5.18)	(4.84)	3.70	0.00	0.00	−2.46[h]	0.00	0
17	3-CN	4.77±0.10 (2)	4.92	(5.47)	2.48	0.00	0.00	−0.58[h]	0.00	0
18	3-NO_2	4.39±0.17 (4)	4.67	(5.87)	2.73	0.00	0.00	−1.65[h]	0.00	0
19	3-CH_3	5.32±0.08 (2)	5.22	(4.85)	3.11[g]	0.00	0.00	−1.16[h]	0.00	0
20	3-OCH_3	5.20±0.03 (2)	5.29	(4.77)	2.81	0.00	0.00	−0.40[h]	0.00	0
21	4-F	5.24±0.08 (2)	5.31	(4.66)	2.87[g]	0.00	0.00	0.00	−0.32[h]	0
22	4-Cl	5.89±0.21 (4)	5.45	(4.18)	3.51[g]	0.00	0.00	0.00	−0.98[h]	0
23	4-Br	5.33±0.02 (3)	5.55	(3.93)	3.73	0.00	0.00	0.00	−1.12[h]	0
24	4-I	3.35±0.12 (2)	(5.55)	3.84	3.96	0.00	0.00	0.00	−1.44[h]	0
25	4-CF_3	4.59±0.04 (2)	(4.64)	5.24	3.68	0.00	0.00	0.00	−2.46[h]	0
26	4-CN	5.35±0.04 (2)	5.84	(5.62)	2.44	0.00	0.00	0.00	−0.58[h]	1
27	4-NO_2	6.44±0.06 (3)	6.54	(6.03)	2.78	0.00	0.00	0.00	−1.65[h]	2
28	4-CH_3	4.48±0.28 (7)	5.01	(4.95)	3.15[g]	0.00	0.00	0.00	−1.16[h]	0
29	4-C_2H_5	5.63±0.11 (2)	5.29	(4.36)	3.59	0.00	0.00	0.00	−1.33[h]	0
30	4-*n*-C_3H_7	5.57±0.12 (4)	5.56	(3.75)	4.11	0.00	0.00	0.00	−1.62[h]	0
31	4-*i*-C_3H_7	4.12±0.28 (5)	(5.54)	3.78	4.11[i]	0.00	0.00	0.00	−1.66[h]	0
32	4-C_6H_5	5.01±0.11 (4)	(4.47)	5.12	4.49	0.00	0.00	0.00	−3.82	0
33	4-OCH_3	4.97±0.11 (2)	5.21	(4.81)	2.82	0.00	0.00	0.00	−0.40[h]	0
34	4-$COCH_3$	5.12±0.18 (2)	5.59	(6.00)	2.42[i]	0.00	0.00	0.00	−0.95[j]	1
35	4-SO_2CH_3	5.81±0.06 (2)	5.23	(8.46)	1.46[i]	0.00	0.00	0.00	−1.86[k]	2
36	2,3-Cl_2	5.54±0.13 (2)	(6.18)	(4.14)	3.75	0.80	−0.98[h]	−0.98[h]	0.00	0
37	2,4-Cl_2	3.91±0.25 (5)	(5.94)	4.35	3.71	0.80	−0.98[h]	0.00	−0.98[h]	0
38	2,5-Cl_2	3.64±0.30 (2)	(6.18)	4.14	3.75	0.80	−0.98[h]	−0.98[h]	0.00	0
39	2,6-Cl_2	5.10±0.15 (3)	(6.29)	5.13	3.03	1.60	−1.96[h]	0.00	0.00	0
40	3,4-Cl_2	3.98±0.10 (4)	(5.68)	3.67	4.25	0.00	0.00	−0.98[h]	−0.98[h]	0
41	3,5-Cl_2	3.55±0.13 (4)	(5.92)	3.46	4.29	0.00	0.00	−1.96[h]	0.00	0
42	2,3-$(CH_3)_2$	5.15±0.08 (2)	(5.89)	4.97	3.40	1.04	−1.16[h]	−1.16[h]	0.00	0
43	2,4-$(CH_3)_2$	5.58±0.05 (2)	(5.62)	5.17	3.38	1.04	−1.16[h]	0.00	−1.16[h]	0
44	2,5-$(CH_3)_2$	4.48±0.11 (2)	(5.89)	4.97	3.40	1.04	−1.16[h]	−1.16[h]	0.00	0
45	3,4-$(CH_3)_2$	5.17±0.06 (2)	(4.95)	4.99	3.65	0.00	0.00	−1.16[h]	−1.16[h]	0
46	3,5-$(CH_3)_2$	4.69±0.04 (2)	(5.21)	4.79	3.67	0.00	0.00	−2.32[h]	0.00	0
47	RH-5992[l]	3.38±0.07 (3)	(6.00)	(3.01)	4.39	0.00	0.00	0.00	−1.33[h]	0
48	RH-2485[l]	3.29±0.11 (2)	(6.69)	(3.48)	3.93	1.04	−1.16[h]	−0.40[h]	0.00	0
49	RH-5849[m]	5.38±0.04 (2)	(5.14)	(5.08)	2.45[n]	0.00	0.00	0.00	0.00	0
50	RH-0345[m]	6.09±0.23 (4)	(5.32)	(4.41)	3.37	0.00	0.00	0.00	−0.98[h]	0

[a] With the mean standard deviation for the number of replications indicated in the parentheses.

[b] The values in parentheses were calculated by eqn (5) and/or eqn (7) but corresponding observed values were not included in the analyses.

[c] For the ascending phase unless parenthesized.

[d] For the descending phase unless parenthesized.

[e] From Ref 9 unless noted.

[f] From Ref 18 unless noted.

[g] Experimentally measured (from Ref 9).

[h] From Ref 21.

[i] Calculated by eqn 12-I of Ref 19.

[j] From Ref 22.

[k] Calculated from the van der Waals radius (Ref 25) according to Ref 18.

[l] A-ring was substituted with 3,5-$(CH_3)_2$.

[m] A-ring was unsubstituted.

[n] Experimentally measured (from Ref 8).

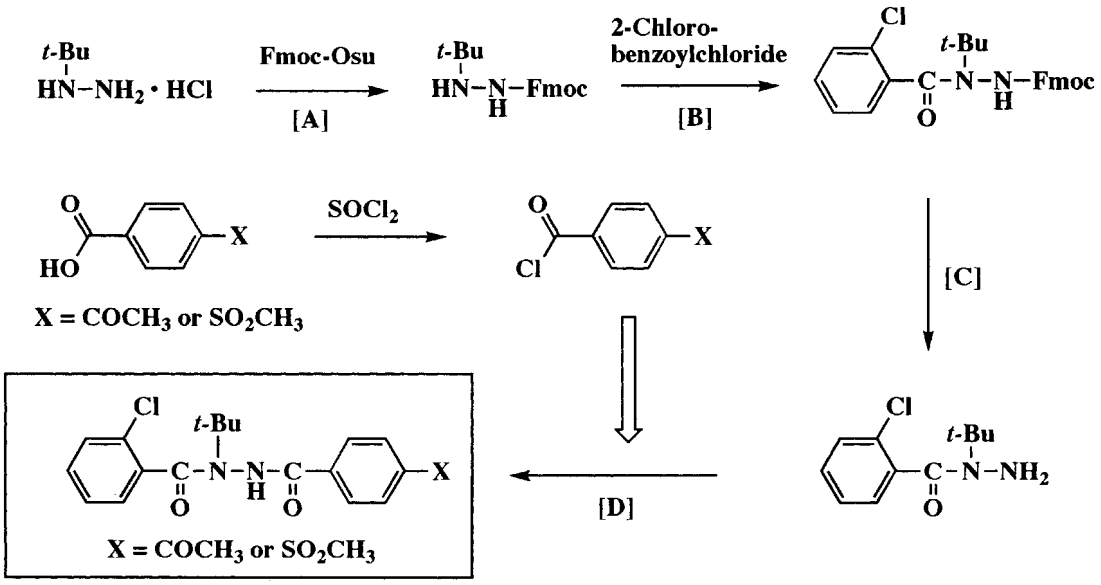


Figure 2. Synthetic scheme for *N-tert*-butyl-*N*-(2-chlorobenzoyl)-*N′*-(substituted benzoyl)hydrazines.

fluorenylmethoxycarbonyl)hydrazine (7.90 g) as a yellowish powder (yield 86.4%).

2.1.3 N-tert-Butyl-N-(2-chlorobenzoyl)hydrazine (Step C)

Piperidine (1.50 g, 0.018 mmol) dissolved in dimethyl formamide (DMF; 10 ml) was added dropwise to *N-tert*-butyl-*N*-(2-chlorobenzoyl)-*N′*-(9-fluorenylmethoxycarbonyl)hydrazine (7.90 g, 17.6 mmol) dissolved in DMF (50 ml). After stirring for 9 h at room temperature, the DMF was removed under reduced pressure. The residue was purified by silica-gel column chromatography with hexane + ethyl acetate (7 + 3 by volume) as eluent. The desired fractions were collected and dried over anhydrous magnesium sulfate. After evaporating the solvent, the residue was crystallized from a mixture of hexane and ethyl acetate to afford yellowish crystals (3.25 g, prism, yield 81.5%).

*2.1.4 N-tert-Butyl-N-(2-chlorobenzoyl)-N′-(4-acetylbenzoyl)hydrazine (Step D) (**34**)*

The 4-acetylbenzoyl chloride obtained from the corresponding benzoic acid (1.1 g, 6.7 mmol) conventionally was dissolved in anhydrous benzene (3 ml). This benzene solution and triethylamine (15 ml) were simultaneously added dropwise to *N*-(2-chlorobenzoyl)-*N-tert*-butylhydrazine (0.45 g, 2.0 mmol) in anhydrous benzene (5 ml) with stirring in an ice bath. After stirring the mixture at room temperature overnight, benzene (100 ml) and ethyl acetate (150 ml) were added to the reaction mixture. The organic layer was washed successively with acidic brine (1 M; 60 ml), three times, saturated sodium bicarbonate (3 × 60 ml), and neutral brine (2 × 60 ml). The organic layer was dried over anhydrous magnesium sulfate, and the solvent was then evaporated. The residue was purified by silica-gel column chromatography with hexane + ethyl acetate (1 + 1 by volume) as eluent, and the main product was manipulated with a mixture of hexane and ethyl acetate to afford *N-tert*-butyl-*N*-(2-chlorobenzoyl)-*N′*-(4-acetylbenzoyl)hydrazine (0.26 g; yield 35.1%). [^{1}H]NMR δ (ppm): 1.62 (9H, s), 2.58 (3H, s), 7.19–7.44 (4H, m), 7.33 (2H, d), 7.88 (2H, d), 8.06 (1H). Analysis; Calcd, C (64.38), H (5.60) and N (7.27); Found, C (64.43), H (5.68), N (7.51). mp 192–195 °C.

*2.1.5 N-tert-Butyl-N-(2-chlorobenzoyl)-N′-(4-methylsulfonylbenzoyl)hydrazine (Step D) (**35**)*

The 4-methylsulfonylbenzoyl chloride (1.26 g, 5.76 mmol) obtained from the corresponding benzoic acid dissolved in anhydrous ether (15 ml) and triethylamine (2 ml) were simultaneously added dropwise to *N-tert*-butyl-*N*-(2-chlorobenzoyl)hydrazine (1.18 g, 5.20 mmol) suspended in anhydrous benzene (5 ml) while stirring in the ice bath. After stirring the mixture at room temperature for three days, ethyl acetate (100 ml) was added to the reaction mixture. The precipitates were removed by filtration. The filtrate was washed successively with hydrochloric acid (1 M 200 ml), saturated sodium bicarbonate (120 ml) and brine (50 ml), then dried over anhydrous magnesium sulfate. After evaporating the solvent, the residue was recrystallized from hexane and ethyl acetate to afford yellowish needles (1.14 g, yield 53.5%). [^{1}H]NMR δ (ppm): 1.62 (9H, s), 3.02 (3H, s), 7.21–7.44 (4H, m), 7.39 (2H, d), 7.87 (2H, d), 8.14 (1H). Analysis: Calcd, C (55.73), H (5.30) and N (6.58); Found, C (55.81), H (5.18), N (6.85). m p 176–177 °C.

2.2. Larvicidal activity

For measurement of larvicidal activity, newly moulted (0–24 h) last-instar (4th) larvae of *L decemlineata* were treated topically in a manner similar to that described

previously.[8,9,17] Various doses of the test compounds (Table 1), were administered in 0.5 µl DMSO with a microsyringe fitted with an applicator (Hamilton, Belgium). At least 20 last-instars were used per dose. Larvae were then fed on freshly cut potato foliage. Mortality counts were made when control larvae had metamorphosed into one-day-old pupae. Preliminary examinations, had shown that the metabolic degradation was insignificant in Colorado potato beetle larvae.[4,5] Larvicidal activity was expressed in terms of pLD_{50}, the log value of the reciprocal of the dose (mmol per insect) required to kill 50% (LD_{50}) of the larvae. All preparations were done at 23 (±2) °C, 65 (±5)% RH and a 16:8 h L:D photoperiod.

2.3 Physicochemical parameters

The most significant combination of physico-chemical parameters affecting larvicidal activity were the molecular hydrophobicity, log *P*, together with hydrogen-bonding and position-specific steric factors. The log *P* value of compounds was either measured experimentally in an octanol-water system or empirically estimated from that of the corresponding benzamide using eqn (1)[9]

$$\begin{aligned}\log P(\mathbf{I}: \mathrm{X_n} = \text{2-Cl}, \mathrm{Y_n}) &= 1.008(\pm 0.040)\log P(\mathrm{Y_n}\text{-benzamides}) \\ &\quad - 0.158(\pm 0.030)\Sigma \mathrm{E_s}^{ortho} \\ &\quad + 1.952\ (\pm 0.046) \\ n = 10,\ s = 0.015,\ r = 0.999,\ F_{2,7} &= 2017.2 \qquad (1)\end{aligned}$$

In this and the following equations, *n* is the number of compounds, *s* is the standard deviation, *r* is the correlation coefficient, and *F* is the value of the ratio between regression and residual variances. The figures in parentheses are the 95% confidence intervals of the regression coefficient. In eqn (1), the $(\sum)\mathrm{E_s}^{ortho}$ value is for the steric effect of *ortho* substituent(s), $\mathrm{E_s}^{ortho}$ being the Taft–Kutter–Hansch value[18] for *ortho* substituents and $\sum$ the sum of the $\mathrm{E_s}^{ortho}$ values for 2,6-disubstituted compounds. The log *P* value of corresponding benzamides was also either experimentally measured or empirically estimated as reported previously.[19] The log *P* value of RH-5849 was experimentally measured,[8] and the values for tebufenozide, methoxyfenozide, and halofenozide were empirically estimated from related compounds as reported.[8,9]

Among various sets of steric parameters, the most reasonable combinations were from the STERIMOL width parameter,[20] B_5 for *ortho*, and the Taft–Kutter–Hansch E_s value[18] for *ortho*, *meta*, and *para* substituents. In some cases it was necessary to replace the B_5 value with E_s value for *ortho*-substituents. For the set of E_s value, E_s(AMD) values were used whenever available. This value is defined experimentally from the acid-catalyzed hydrolytic rate constants of *ortho*-substituted benzamides[21] and from the extended Hammett–Taft analysis of the quaternization rate constant of *ortho*-substituted dimethylanilines.[22] A composite set of the Taft–Kutter–Hansch E_s including the E_s(AMD) value has been shown to represent the steric effect of substituents better than the regular set of the Taft–Kutter–Hansch E_s value often.[23,24] The E_s value of the SO_2CH_3 group was estimated according to Kutter and Hansch[18] from its 'effective' van der Waals dimension (=2.23) proposed by Charton[25] for the SO_3-group. The E_s value is defined so that the bulkier the substituent, the more negative the value.

For hydrogen-bond acceptor substituents at the *para* position, a site-specific effect represented by an indicator variable, HB, was considered in the analysis.[26] To substituents with the hydrogen-accepting site at the β-position from the benzene ring, HB values were assigned so that HB = 0 for OMe, HB = 1 for CN and $COCH_3$, and HB = 2 for NO_2 and SO_2CH_3. Relevant parameter values are listed in Table 1. The reference point of each of the steric parameter sets is shifted to H, so that the Δ means the difference from the corresponding parameter value for H.

3 RESULTS

3.1 Larvicidal activity

To obtain reliable larvicidal activity values in terms of pLD_{50} (mmol per insect), we repeated the bioassay at least twice. The activity value for the unsubstituted compound **1** (H) was estimated to be 5.46 (±0.15) as the average of five replications. Each replicated pLD_{50} value is observed within the range of the average ± 0.30 (standard deviation). The averaged pLD_{50} value with the standard deviation is listed in Table 1.

Introduction of a substituent into the *ortho* position of the B-ring mostly enhances the activity of the unsubstituted compound (**1**) unless it leads to a log *P* value higher than 3.1. Thus, whereas CF_3 (**6**), CH_3 (**8**), OCH_3 (**10**) and SCH_3 (**11**) groups elevate the activity 3-to 8-fold, the I (**5**) and Ph (**9**) substituents lower it to 1/3 of that of unsubstituted **1**. At the *meta* position, substituents other than lower halogens [F (**12**), Cl (**13**), and Br (**14**)] are unfavorable to the activity. Especially striking is the effect of I (**15**) and CF_3 (**16**), which decrease the activity of compound **1** to about 1/60 and 1/200, respectively. This strikingly unfavorable effect seems to appear above a threshold log *P* value of about 3.7. Most *para*-substituted compounds are either almost equipotent to or less potent than the unsubstituted **1**. Exceptions are compounds with hydrogen-bond-accepting substituents such as NO_2 (**27**) and SO_2CH_3 (**35**), which are very favorable to activity at the *para* position (6- to 10-fold enhancements). As with *ortho* and *para* substituents, there seems to be a threshold in the log *P* value at about 4.0, above which the activity is drastically decreased in such compounds as I (**24**), *i*-C_3H_7 (**31**), and C_6H_5 (**32**).

The effect of disubstitutions was examined only for Cl and CH_3 substituents at various positions. While 2,3-Cl_2 (**36**) and 2,4-$(CH_3)_2$ (**43**) compounds are

almost equipotent to, others are less potent than, the unsubstituted **1**. In the corresponding positional isomeric pairs, dimethyl compounds with lower log *P* values are more active than the dichloro compounds, except for the 2,3-disubstituted pair. This may indicate that the log *P* value of disubstituted compounds examined here is mostly above the threshold. It might be located at about 3.0, but the disubstitution pattern itself may possibly be involved in the location of the threshold.

Although the above observations are not straightforward, they at least suggest a great importance for the molecular hydrophobicity in the structure–activity pattern, such as the position-specific threshold log *P* values. Thus, we started to examine structure–activity relationships at each of the mono-substituted positional isomers with log *P* values that were considered to be lower than the threshold.

3.2 QSAR analyses

For *ortho*-substituted compounds, omitting compounds **5** (2-I) and **9** (2-C_6H_5), which are above the log *P* threshold of 3.1, eqn (2) was formulated.

$$\begin{aligned} \mathrm{pLD_{50}} &= 0.797(\pm 0.641)\log P \\ &\quad + 0.241(\pm 0.276)\Delta \mathrm{B_5}^{ortho} + 3.452(\pm 1.767) \\ n &= 9,\ s = 0.239,\ r = 0.829,\ F_{2,6} = 6.604 \end{aligned} \quad (2)$$

The $\Delta B_5{}^{ortho}$ term in eqn (2) is justified only at the 92.4% level, but significant over the 95% level when other positional isomers are analyzed together, as shown later in eqn (5). The use of other steric parameters gave poorer correlations.

For *meta*-substituted compounds, not including compounds **15** (3-I) and **16** (3-CF_3), for which log $P > 3.7$, eqn (3) was formulated as the best.

$$\begin{aligned} \mathrm{pLD_{50}} &= 1.289(\pm 0.491)\log P \\ &\quad + 0.714(\pm 0.373)\mathrm{E_s}^{meta} + 2.042(\pm 1.368) \\ n &= 8,\ s = 0.188,\ r =; 0.954,\ F_{3,6} = 25.347 \end{aligned} \quad (3)$$

Para-substituted compounds were analyzed to give eqn (4), excluding over-threshold compounds **24** (I), **31** (*i*-C_3H_7) and **32** (C_6H_5) in which in addition to the threshold condition of log $P > 4.0$, it is necessary for $-\mathrm{E_s(AMD)} > 1.65$ to discriminate between **30** (*n*-C_3H_7) and **31** (*i*-C_3H_7).

$$\begin{aligned} \mathrm{pLD_{50}} &= 0.720(\pm 0.675)\log P \\ &\quad + 0.453(\pm 0.518)\mathrm{E_s}^{para} + 0.965(\pm 0.633)\mathrm{HB} \\ &\quad + 3.265(\pm 1.961) \\ n &= 13, s = 0.394, r = 0.765, F_{3,10} = 4.226 \end{aligned} \quad (4)$$

In eqn (4), HB is an indicator variable for hydrogen-bond acceptors at the β-position from the ring which were defined above. Even though the $\mathrm{E_s}^{para}$ term is justified only at the 92% level in eqn (4), it is significant over the 95% level when other positional isomers are analyzed, as shown below in eqn (5).

Since the coefficients of the log *P* term are not far from each other in eqns (2)–(4), overlapping within the 95% confidence intervals, they were combined to give eqn (5) for the compounds included in the 'ascending' phase of the activity in terms of log *P*.

$$\begin{aligned} \mathrm{pLD_{50}} &= 0.887(\pm 0.363)\log P \\ &\quad + 0.395(\pm 0.240)\Delta \mathrm{B_5}^{ortho} \\ &\quad + 0.435(\pm 0.368)\mathrm{E_s}^{meta} \\ &\quad + 0.650(\pm 0.313)\mathrm{E_s}^{para} \\ &\quad + 1.088(\pm 0.389)\mathrm{HB} + 2.968(\pm 1.053) \\ n &= 28, s = 0.328, r = 0.842, F_{5,22} = 10.678 \end{aligned} \quad (5)$$

At this stage, combinations of various steric parameters for various positional substituents were thoroughly examined. Equation (5) was found to be best not only statistically but also predictively for the activity of compounds not included in the analysis. The $\mathrm{pLD_{50}}$ values of 28 compounds calculated by eqn (5) are listed in Table 1.

Although the log *P* threshold value is not clear and the number of compounds is insufficient to establish precise correlations, examination of the structure/activity relationships of the disubstituted derivatives gave useful information. We found that a 'tolerable' correlation equation (eqn (6)) could be derived for 10 disubstituted compounds (**37–46**). The 2,3−Cl_2 compound (**36**) was not included in eqn (6), because its activity is much higher than that expected from the equation including it.

$$\begin{aligned} \mathrm{pLD_{50}} &= -2.115(\pm 1.029)\log P \\ &\quad - 0.537(\pm 0.559)\mathrm{E_s}^{meta} \\ &\quad - 0.902(\pm 0.709)\mathrm{E_s}^{para} \\ &\quad + 11.343(\pm 3.314) \\ n &= 10,\ s = 0.368,\ r = 0.909,\ F_{3,6} = 9.544 \end{aligned} \quad (6)$$

In eqn (6), the $\mathrm{E_s}^{meta}$ term for the *meta* substituent is significant at the 94.2%, slightly below the 95% level. Among others, the combination of $\Delta \mathrm{V_w}^{meta}$ and $\mathrm{E_s}^{para}$ parameters gave a very similar correlation ($s = 0.368$, $r = 0.909$, $F_{3,6} = 9.567$), ΔV_w being the increment in the van der Waals volume defined by Bondi.[27] We prefer eqn (6), because it is simpler, using a single type of steric parameter. The addition of $\Delta \mathrm{B_5}^{ortho}$ or other types of steric parameter term in eqn (6) did not improve the correlation. The situation is not incompatible with steric parameter sets used in eqns (3) and (4), although the sign is reversed.

Encouraged by the above finding, and assuming that the origin of the negative sign of the log *P* term in eqn (6) is common with that of the negative dependence on the log *P* value above the threshold in mono-substituted derivatives, we combined compounds **5** (2-I), **9** (2-C_6H_5), **15** (3-I), **24** (4-I), **31** (4-*i*-C_3H_7) and **32** (4-C_6H_5) into eqn (6). Thus, eqn (7) was formulated representing the 'descending' phase of the

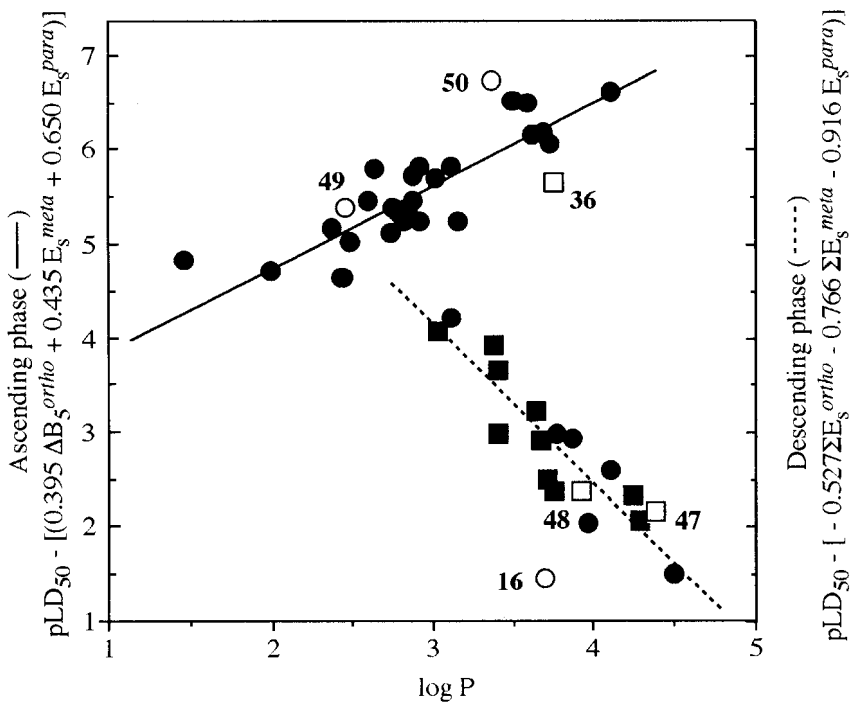


Figure 3. Partial relationship between pLD_{50} and log *P*. (●) Mono-substituted compounds included in either eqn (5) or eqn (7). (■) Di-substituted compounds included in eqn (7). (□) Predicted value of the 2,3-Cl_2 compound (**36**), RH-5849 (**49**) and halofenozide (**50**) by eqn (5) for ascending phase. (○) Predicted value of the 3-CF_3 compound (**16**), tebufenozide (**47**) and methoxyfenozide (**48**) by eqn (7) for descending phase.

activity in terms of the molecular hydrophobicity.

$$\begin{aligned} pLD_{50} = &-1.695(\pm 0.744)\log P \\ &- 0.527(\pm 0.320)\Sigma E_s^{ortho} \\ &- 0.766(\pm 0.476)\Sigma E_s^{meta} \\ &- 0.916(\pm 0.408)E_s^{para} + 9.226(\pm 2.578) \\ n = 16,\ s = 0.373,\ r = 0.885,\ &F_{4,11} = 9.982 \end{aligned} \quad (7)$$

In eqn (7), $\sum$ means the sum of parameter values for 2,6- and 3,5-disubstitutions. Using ΔB_5 instead of E_s for *ortho* substituents, another significant equation was formulated, although the correlation was slightly worse ($s = 0.415$, $r = 0.856$) than that of eqn (7). By the same reason for the selection of eqn (6), however, eqn (7) was taken to represent the situation of the compounds in the 'descending' phase. Compound **16** (3-CF_3) was not included in eqn (7), because its activity is too low to be predicted by structure–activity pattern of other compounds in the 'descending' phase. Another thing to be mentioned here is that the E_s value of the Ph group in compounds **9** and **32** is not that of E_s(AMD) but that estimated in terms of the half-width of the benzene ring. The use of E_s(AMD) value estimates the activity much higher. Equation (7) also indicates that the steric effects of multiple substitutions on the activity are almost additive. The pLD_{50} values for those in the 'descending' phase calculated by eqn (7) are listed in Table 1.

4 DISCUSSION

The above QSAR analyses are indicative of the fact that there are two phases in the structure–activity pattern of this series of compounds: activity ascending and descending phases depending on the molecular hydrophobicity. The situation is illustrated in Fig 3. The boundary differentiating between ascending and descending series is not clear when the entire series of compounds is considered together. The boundary is position-specific, being located at the highest log *P* region for the *para* and lowest for the *ortho* position. The disubstitution may also be a factor differentiating the phases. It may 'inherently' make the compounds belong to the descending phase.

There are two outliers not included in correlations, ie the 3-CF_3 (**16**) and the 2,3-Cl_2 (**36**) compounds. The very low activity of compound **16** (3-CF_3) may not be too accurate. The activity (5.54) of compound **36** is rather close to the activity calculated as the one of the ascending series (6.18 calculated from eqn (5). The reason is not clear at the moment. Not only dichloro and dimethyl compounds but other combinations with various substitution patterns should also be included in the analyses to understand the mechanism for differentiating the ascending and descending phases, especially in disubstituted compounds.

Despite the above uncertainties, the biphasic QSAR relationships seem to predictively rationalize the difference in the activity spectrum between two sets of compounds not included in the analysis: one is tebufenozide (RH-5992: **47**) and methoxyfenozide (RH-2485: **48**) and the other is RH-5849 (**49**) and halofenozide (RH-0345: **50**). The two compounds **49** and **50** are quite active against Colorado potato beetle larvae. Their activity values are close to or not very far from those calculated by eqn (5), but (much) higher than those calculated by eqn (7), as shown in Table 1. Equation (5) takes into account, however, only the effects of substituents attached to the B-ring, except for that of the molecular log *P*. Differing from compounds included in eqn (5), there is no substituent

in the A-ring in these two compounds **49** and **50**. The fact that their activity values are close to those from eqn (5) suggests that the structural requirements of the A-ring for activity against Coleoptera are not very rigid. There is no substituent on the B-ring in RH-5849 (**49**), and only the 4-Cl substituent in compound **50**, so that the log *P* values are not so high and are reasonably located below the threshold.

On the other hand, tebufenozide (**47**) and methoxyfenozide (**48**), not included in the analyses, are not so active against the Colorado potato beetle larvae. Their A-ring is 3,5-$(CH_3)_2$-substituted, while the B-ring is 4-C_2H_5 (**47**) and 2-CH_3-3-OCH_3-substituted (**48**). Their log *P* values are relatively high and reasonably above the threshold. Their low activity against the Colorado potato beetle larvae is much better 'predicted' by eqn (7) for the descending series of compounds than those calculated by eqn (5) as shown in Table 1. This is in support of the above suggestion that the effect of the A-ring substituents is not rigid. Thus, one of the origins of the difference between the activity spectra of the above two sets of compounds seems to be due to the difference in the molecular log *P* value as to whether it is above or below the threshold.

The activity values most poorly predicted in eqn (5) are those of *para*-substituted compounds in which substituents are hydrogen-accepting, such as CN (**26**), NO_2 (**27**), $COCH_3$ (**34**), and SO_2CH_3 (**35**). The hydrogen-accepting NO_2 (**27**) and SO_2CH_3 (**35**) compounds are, in fact, among the most active compounds in this series. The participation of the electron-withdrawing effect of *para* substituents is unlikely, because the effect of electron-releasing substituents such as C_2H_5 (**29**) and *n*-C_3H_5 (**30**) is about the same as that of SO_2CH_3 (**35**), and also because no electronic effect seems to operate with *ortho* and *meta* substituents. There are a number of examples in which hydrogen-accepting substituents enhance the activity.[26] The hydrogen-bonding interaction occurs at the substituent site but not at the 'functional group site'. This type of hydrogen-bonding effect can be expressed by the indicator variable.[26] The regression coefficient of the indicator variable term may be the ratio of the molarity of hydrogen-donor group at the receptor site relative to that in the octanol phase.[26]

There are also examples in which the indicator variable values for hydrogen-accepting substituents are taken to be unity, or two, depending upon the number of hydrogen-bonding sites.[28] Probably because the hydrogen-bonding interaction is quite sensitive to the directionality of interaction partners, the variable was not needed for the OCH_3 (**33**) compound where the site is located at the α-position away from the ring. The fact that the largest deviations were observed in this hydrogen-acceptor compound indicates that the above assignments are still not sufficient. Additional compounds with substituents capable of hydrogen-bonding at the *para* position are needed for further measurements and analyses, because the exploration of similar type of substituents at this position could lead to highly potent and selective analogs against coleopterous larvae.

Equation (5) indicates that the coleopterous activity of this series of compounds increases with increasing molecular hydrophobicity up to the threshold position. The steric effect of the *ortho* substituents parameterized by ΔB_5 is such that the greater the 'maximal' width, the greater the activity. On the other hand, the greater the steric bulk of *meta* and *para* substituents in terms of $-E_s$, the lower the activity. For the *para* substituents, a boundary was also considered for the value of $-E_s$ above which the structure–activity pattern shifts to the 'descending' phase in terms of the log *P* dependence.

Even though the threshold log *P* value depends upon substituent positions and substitution patterns, compounds beyond the threshold are analyzable as a set quantitatively, as shown in eqn (7). Of course, in this phase, the greater the hydrohobicity, the lower the activity. The sign of the *ortho* steric term (E_s) in eqn (7) is negative, indicating that the bulkier substituents are favorable to activity, similar to that in eqn (5). The favorable steric effect of *ortho* substituents throughout the two phases may indicate an inhibitive function against hydrolytic degradation and/or twisting conformations of the CONH moiety favorable to the receptor interactions. The signs of the *meta* and *para* steric (E_s) terms in eqn (7) are opposite to those in eqn (5), which seems to mean that the bulkier the substituents, the higher the activity. Thus, the interaction pattern with which *meta* and *para* substituents are involved differs between compounds in 'ascending' and 'descending' phases. The log *P* and *meta* and *para* steric terms are associable/collaborative in governing activity variations.

In eqn (7) for the 'descending' phase, the E_s value for the half-width of the ring plane, not E_s(AMD), was used for the 2-C_6H_5 (**9**) and 4-C_6H_5 (**32**) compounds. The use of E_s (half-width) value was much better than that of experimentally derived E_s(AMD) in formulating eqn (7). The ring plane of the phenyl group on the benzene ring would be more twisted than in the intermediate state of the *ortho*-phenylbenzamide hydrolysis, so that the use of E_s (half-width) in the descending phase seems to indicate that the two rings are more or less perpendicular.

The dependence of activity on the log *P* value of this series of compounds is certainly not simple. There seem to be optimum log *P* values multiply depending upon the substituent positions (and substitution patterns) so that neither parabolic nor bilinear formulation for the entire set of compounds is possible. The biphasic dependence should indicate the participation of a great number of transfers through barriers between lipid and aqueous phases during transport from the application to the action site. In our earlier studies with lepidopterous larvae using a set of compounds very similar to those in the

present study, the QSAR correlation equation included only a linear term of log *P* not depending on the substituent position. The optimum log *P* would appear to be higher than that of the most hydrophobic compound included in the series (compound **32**: log $P = 4.49$).[9,15] In this case, the dependence of the activity on log *P* was similar to that in one-step partitioning.[29] Thus, the matrix structure of the cuticular tissue of lepidopterous larvae could be expected to be 'simpler' than that of coleopterous larvae. In fact, the number of lamellar layers in the cuticle of larvae of lepidopterous *S exigua* is around 70, whereas in coleopterous *L decemlineata* larvae it is 120–150.[30,31]

In conclusion, the toxicity of dibenzoylhydrazine analogs against Colorado potato beetle larvae was shown to be governed very significantly by the optimum molecular hydrophobicity, log *P*, position-specifically. The optimum value of log *P* is in the range of 3.0 (*ortho*) to 4.0 (*para*). These optimum log *P* values would be much lower than those for lepidopterous larvae such as rice stem borers and beet armyworms. The significant difference in the log *P* dependence between QSARs for lepidopterous and coleopterous larvae was believed to be the main source of the difference in the insecticidal spectra of certain compounds in this series between the two families. Hydrogen-accepting groups such as NO_2 and SO_2CH_3 at the *para* position of the ring in the *N*-unsubstituted benzamide moiety increase the larvicidal activity to the highest extent against Colorado potato beetle larvae. Further projects designing insect-selective analogs will include systematic syntheses and bioassays of *para*- and multi-substituted compounds as well as their QSAR analyses.

ACKNOWLEDGEMENTS

This investigation was supported, in part, by a Grant-in-Aid for the Scientific Research from the Ministry of Education, Science, and Culture of Japan (10161207). The authors acknowledge the kind gifts of ecdysone agonists by Dr G R Carlson (Rohm and Haas Research Laboratories, PA, USA). Dr Guy Smagghe is a post-doctoral research fellow of the National Fund of Scientific Research of Belgium, Brussels.

REFERENCES

1 Heller JJ, Mattioda H, Klein E and Sagenmuller A, Field evaluation of RH-5992 on lepidopterous pests in Europe, in *Proc Brighton Crop Prof Conf – Pests and Diseases*. pp 59–65 (1992).

2 Hsu AC-T, Fujimoto TT and Dhadialla TS, Structure–activity study and conformational analysis of RH-5992, the first commercialized nonsteroidal ecdysone agonist, in *Phytochemicals for Pest Control*, ed by Hedin PA, Hollingworth RM, Masler EP Miyamoto J and Thompson DG, *ACS Symp* Ser No 658, American Chemical Society, Washington, DC. pp 206–219 (1997).

3 Le DP, Thirugnanam M, Lidert Z, Carlson GR and Ryan JB, RH-2485: A new selective insecticide for caterpillar control, in *Proc Brighton Crop Prot Conf – Pests and Diseases*. pp 481–485 (1996).

4 Smagghe G and Degheele D, The significance of pharmacokinetics and metabolism to the biological activity of RH 5992 (tebufenozide) in *Spodoptera exempta*, *Spodoptera exigua*, and *Leptinotarsa decemlineata*. *Pestic Biochem Physiol* **49**:224–234 (1994).

5 Smagghe G and Degheele D, Metabolism, pharmacokinetics, and toxicity of the first nonsteroidal ecdysteroid agonist RH-5849 to *Spodoptera exempta* (Walker), *Spodoptera exigua* (Hübner), and *Leptinotarsa decemlineata* (Say). *Pestic Biochem Physiol* **46**:149–160 (1993).

6 RohMid LLC, RH-0345, turf and ornamental insecticide. *Tech Info Bull*, p 10 (1996).

7 Dhadialla TS, Carlson GR and Le DP, New insecticides with ecdysteroidal and juvenile hormone activity. *Ann Rev Entomol* **43**:545–569 (1998).

8 Oikawa N, Nakagawa Y, Nishimura K, Ueno T and Fujita T, Quantitative structure–activity analysis of larvicidal 1-(substituted benzoyl)-2-benzoyl-1-*tert*-butylhydrazines against *Chilo suppressalis*. *Pestic Sci* **41**:139–148 (1994).

9 Oikawa N, Nakagawa Y, Nishimura K, Ueno T and Fujita T, Quantitative structure–activity studies of insect growth regulators. X. Substituent effects on larvicidal activity of 1-*tert*-butyl-1-(2-chlorobenzoyl)-2-(substituted benzoyl)hydrazines against *Chilo suppressalis* and design synthesis of potent derivatives. *Pestic Biochem Physiol* **48**:135–144 (1994).

10 Nakagawa Y, Soya Y, Nakai K, Oikawa N, Nishimura K, Ueno T, Fujita T and Kurihara N, Quantitative structure–activity studies of insect growth regulators. XI. Stimulation and inhibition of *N*- acetylglucosamine incorporation in a cultured integument system by substituted *N*-*tert*-butyl- *N*,*N*′-dibenzoylhydrazines. *Pestic Sci* **43**:339–345 (1995).

11 Nakagawa Y, Shimizu B, Oikawa N, Akamatsu M, Nishimura K, Kurihara N, Ueno T and Fujita T, Three-dimensional quantitative structure–activity analysis of steroidal and dibenzoylhydrazine-type ecdysone agonists, in *Classical and Three-Dimensional QSAR in Agrochemistry*, ed by Hansch C and Fujita T, *ACS Symp* Ser No 606, American Chemical Society, Washington, DC. pp 288–301 (1995).

12 Nakagawa Y, Hattori K, Shimizu B, Akamatsu M, Miyagawa H and Ueno T, Quantitative structure–activity studies of insect growth regulators XIV. Three-dimensional quantitative structure–activity relationship of ecdysone agonists including dibenzoylhydrazine analogs. *Pestic Sci* **53**:267–277 (1998).

13 Hansch C and Fujita T, ρ-σ-π Analysis. A method for the correlation of biological activity and chemical structure. *J Am Chem. Soc* **86**:1616–1626 (1994).

14 Cramer III RD, Patterson DE and Bunce JD, Comparative molecular field analysis (CoMFA). 1. Effect of shape on binding of steroids to carrier proteins. *J Am Chem Soc* **110**:5959–5967 (1988).

15 Smagghe G, Nakagawa Y, Carton B, Mourad AK, Fujita T and Tirry L, Comparative ecdysteroid action of ring-substituted dibenzoylhydrazines in *Spodoptera exigua*. *Arch Insect Biochem Physiol* **41**:42–53 (1999).

16 Hsu AC-T, 1,2-Diacyl-1-alkylhydrazines. A new class of insect growth regulators, in *Synthesis and Chemistry of Agrochemicals II*, ed by Baker DR, Fenyes JG and Moberg WK, *ACS Symp* Ser No 443, American Chemical Society, Washington, DC. pp 478–490 (1991).

17 Smagghe G and Degheele D, Action of a novel nonsteroidal ecdysteroid mimic, tebufenozide (RH-5992), on insects of different orders. *Pestic Sci* **42**:85–92 (1994).

18 Kutter E and Hansch C, Steric parameters in drug design. Monoamine oxidase inhibitors and antihistamines. *J Med Chem* **12**:647–652 (1969).

19 Sotomatsu T and Fujita T, Octanol/water partition coefficient of

ortho-substituted aromatic solutes. *J Pharm Sci* **82**:776–781 (1993).
20 Verloop A, The STERIMOL approach: Further development of the method and the new applications, in *Pesticide Chemistry, Human Welfare and Environment*, Vol 1, ed by Miyamoto J and Kearney PC, Pergamon Press, Oxford. pp 339–344 (1983).
21 Sotomatsu T and Fujita T, Analysis of *ortho* effects with a steric parameter defined by acidic hydrolysis rate of *ortho*-substituted benzamides. *Bull Chem Soc Jpn* **65**:2343–2348 (1992).
22 Sotomatsu T, *The analysis of the ortho effect*, Ph D thesis, Faculty of Agriculture, Kyoto University, Kyoto (1989).
23 Sotomatsu T and Fujita T, The QSAR applications of a new steric parameter set for aromatic substituent defined by the acidic hydrolysis rate of *ortho*-substituted benzamides. *Quant Struct–Act Relat* **9**:295–301 (1990).
24 Nakao K, Shimizu R, Kubota H, Yasuhara M, Hashimura Y, Suzuki T, Fujita T and Ohmizu H, Quantitative structure–activity analyses of novel hydroxyphenylurea derivatives as antioxidants. *Bioorg Med Chem* **6**:849–868 (1998).
25 Charton M, The upsilon steric parameter – definition and determination, in *Steric Effects in Drug Design, Topics in Current Chemistry* Vol 114, ed by Charton M and Motoc I, Springer-Verlag, Berlin. pp 57–91 (1983).
26 Fujita T, Nishioka T and Nakajima M, Hydrogen-bonding parameter and its significance in quantitative structure–activity studies. *J Med Chem* **20**:1071–1081 (1977).
27 Bondi A, van der Waals volumes and radii. *J Phys Chem* **68**:441–451 (1964).
28 Yamagami C, Takao N and Fujita T, Hydrophobicity parameter of diazines. III: Relationship of partition coefficients of monosubstituted diazines and pyridines in different partitioning systems. *J Pharm Sci* **82**:155–161 (1993).
29 Pennistone JT, Beckett L, Bentley DL and Hansch C, Passive permeation of organic compounds through biological tissue: a non-steady-state theory. *Mol Pharmacol* **5**:331–341 (1969).
30 Smagghe G, Viñuela E, Budia F and Degheele D, *In vivo* and *in vitro* effects of the nonsteroidal ecdysteroid agonist tebufenozide on cuticle formation in *Spodoptera exigua:* an ultrastructural approach. *Arch Insect Biochem Physiol* **32**:121–134 (1996).
31 Smagghe G, Viñuela E, Van Limbergen H, Budia F and Tirry L, Nonsteroidal moulting hormone agonists: Effect on protein synthesis and cuticle formation in Colorado potato beetle larvae. *Entomol. Exp App* (in press).